AD_____

Award Number: DAMD17-00-1-0122

TITLE: Novel Vectors for Dendritic Cell Transduction

PRINCIPAL INVESTIGATOR: Theresa V. Strong, Ph.D.

CONTRACTING ORGANIZATION: The University of Alabama at Birmingham
Birmingham, Alabama 35294-0111

REPORT DATE: June 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2001	Annual (1 Jun 00 - 31 May 01)

4. TITLE AND SUBTITLE
Novel Vectors for Dendritic Cell Transduction

5. FUNDING NUMBERS
DAMD17-00-1-0122

6. AUTHOR(S)
Theresa V. Strong, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Alabama at Birmingham
Birmingham, Alabama 35294-0111

E-Mail: theresa.strong@ccc.uab.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

Dendritic cells (DCs) show promise for cancer immunotherapy due to their critical role in mediating immune response. Development of an optimal DC transduction protocol for tumor antigen presentation would represent a significant advancement in DC-based vaccination strategies. We are evaluating novel vector systems for dendritic cell transduction. Replicative RNA transcripts based on the alphavirus family are noninfectious vectors capable of directing cytoplasmic replication and high level transgene expression upon entry into the cell. We hypothesize that the augmented expression afforded by the replicative RNA system will effectively stimulate T cells. Further, we hypothesize that the cytopathic effects induced by these vectors will enhance the DC-based immune response as suggested by the "danger" model. A second approach uses adenoviral vectors to targeted to CD40 on DCs via a bispecific antibody, which we anticipate will promote maturation of the DCs. The target antigen we will evaluate is carcinoembryonic antigen (CEA). Animals will be immunized with the genetically modified DCs and induction of anti-CEA immune responses will be measured. Tumor challenge and tumor therapy experiments will be performed using a syngeneic adenocarcinoma cell line which expresses human CEA (MC38-CEA-2), and toxicity will be monitored. Both nontransgenic and CEA-transgenic mouse models will be used.

14. SUBJECT TERMS	15. NUMBER OF PAGES
dendritic cells, immunotherpay, antigen	7
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Novel Vectors for Dendritic Cell Transduction
DAMD17-00-1-0122
Principal Investigator: Theresa V. Strong, Ph.D.
Annual Report June 1, 2000 – May 31, 2001

INTRODUCTION:

Cancer immunotherapy approaches aim to enhance the cellular immune response against tumor antigens. Although cytolytic T cells specific for tumor antigens can be isolated from tumor-bearing individuals, it is clear that immune system fails to produce effective antitumor immunity. In recent years, dendritic cells (DCs) have received much attention as their critical role in the elicitation of immune response has been appreciated. Preclinical studies and initial clinical trials using these cells for tumor antigen presentation have produced some encouraging results, however, gene transfer technology for DCs has not yet been optimized. In this project, we are evaluating two novel vector systems for gene transfer into DC. Naked RNA has been shown to mediate gene transfer into dendritic cells and we hypothesize that the use of replicative RNA will enhance transgene expression and improve tumor antigen presentation. Further, we believe that the cytopathic nature of gene expression using this system will act as a "danger" signal and activate a more effective immune response. As a second gene transfer system we are studying a targeted adenoviral vector developed by our collegues in the Gene Therapy Center at the University of Alabama at Birimingham (1). This adenoviral vector is specifically targeted to the CD40 molecule present on dendritic cells. Studies with human dendritic cells demonstrated that this modified virus efficiently transduces DCs and leads to DC maturation. We hypothesize that these features will render the vector capable of inducing a strong antitumor immune response. As a model system to evaluate these vectors, we will deliver the human CEA gene to murine dendritic cells and evaluate the ability of these vectors to break immunological tolerance, induce a CEA-specific immune response and mediate an effective antitumor immune response. The specific aims of this project are:

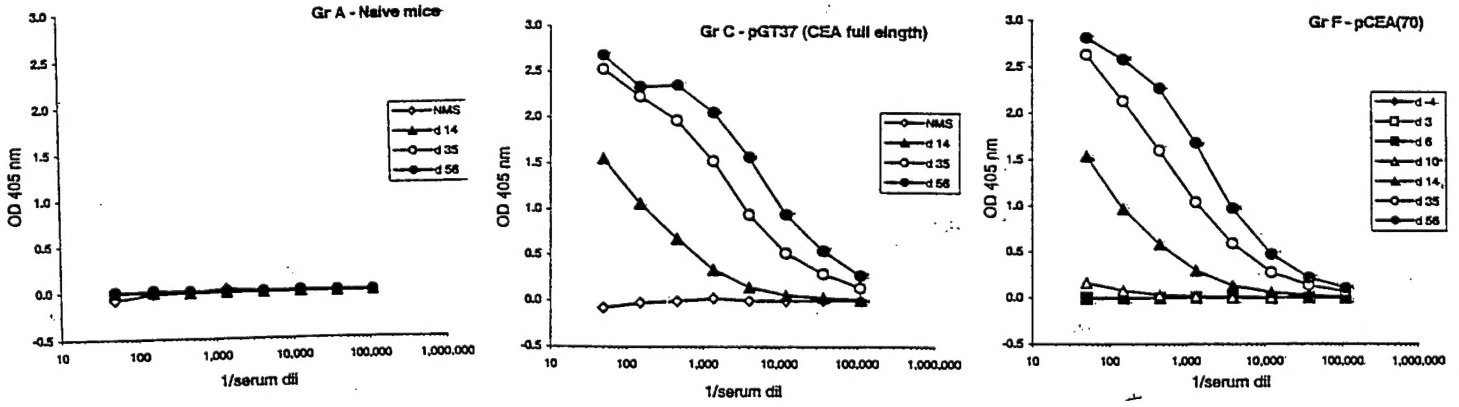
1. To evaluate the ability of replicative RNA vectors encoding CEA to transfect dendritic cells ex vivo, and elicit an antitumor immune response in a CEA transgenic mouse model of adenocarcinoma.

2. To use a bispecific antibody to produce a CD40-targeted adenovirus encoding CEA, and to evaluate its specificity in transducing dendritic cells and efficacy in inducing an antitumor immune response in a CEA transgenic mouse model.

BODY:

Production of mouse DC for transfection / transduction: We evaluated several protocols for the isolation and culture of mouse dendritic cells (DC). We initially attempted DC isolation from mouse spleens following collagenase D treatment and evaluated the use of magnetic beads with anti-CD11c to isolate the DC precursors. Cells were then grown in IL-4 and GM-CSF. After 7 days in culture, the proportion dendritic cells was assessed by flow cytometry. This approach resulted in disappointing yields, so we next tried to increase the DC population *in vivo* by injection of a plasmid DNA encoding the flt-3 ligand (pNGVL3-Flex). Although this resulted in increased spleen size, the yield of DC after in vitro culture was again disappointing. Finally, we have developed a protocol based on isolation of DC precursors from mouse bone marrow. Mouse bone marrow is flushed and cultured with GM-CSF and IL-4. After 6 to 7 days in culture the DCs are matured using 1μg/ml LPS. This protocol consistently yields adequate DC for our experiments.

Production and validation of a truncated CEA: We produced a vector encoding a shortened version of the CEA protein. This CEA cDNA is internally deleted to remove the second of three repetitive segments, joining repeats I and III. Based on our previous studies, we felt this construct would encode all of the necessary regions to mediate anti-CEA immunity capable of mediating tumor rejection. This construct should prove beneficial in requiring synthesis of a shorter RNA transcript from the replicative RNA vectors. It also allows for the production of fusion proteins. Although not proposed in our original grant, this construct will allow us the flexibility to include moieties to enhance immune response (such as GM-CSF) and may further improve the efficacy of these vectors. We evaluated whether the shortened version of CEA was comparable to the full length version in inducing anti-CEA immunity and tumor rejection. For these studies, immunization with DNA encoding the shortened CEA (CEAp70) was compared to the full length construct. Mice were immunized by intramuscular administration of the nucleic acid in phosphate buffered saline. The immunization schedule was a 50 μg dose once every two weeks for three doses. Antibody response showed that the two CEA-encoding plasmids produced comparable induction of antibodies to CEA:



ELISA analysis of CEA-specific antibodies in the serum of immunized animals. NMS-normal mouse sera.

In addition, the ability of the immunized animals to reject tumor was comparable. When challenged with a syngeneic cell line expressing CEA, 0/10 animals immunized with pGT37 (full length) and 1/10 animals immunized with pCEA-70 developed tumors, while 10/10 naïve animals, and 10/10 animals injected with an irrelevant plasmid developed tumors.

Optimization of T cell immune response assays: In contrast to lymphoproliferative assays, which have been less sensitive and have produced more variable results, we have found the cytokine release assay to be a reliable and consistent assay for assessing T cell response to immunization with nucleic acids encoding CEA. In this assay, splenocytes from immunized animals are cultured with purified human CEA. Supernatants from the cultured cells are used in an ELISA-based assay for detection of cytokine release. Using the Biosource Cytoscreen kit for mouse interferon-gamma, we found the full length CEA (pGT37) and the shortened CEA (pCEA-70) to be comparable in their ability to induce T cell response as measured by interferon-gamma release. Mice were immunized intramuscularly three times, 50 μg/dose with plasmid DNA encoding the CEA cDNAs. Immunization with the empty plasmid, NGVL3, served as the negative control. :

	Medium	Ova	CEA	ConA
pNGVL3	2.9	2.1	2.6	423
pGT37	5.8	2.0	28.3	815
pCEA70	3.0	3.4	70.9	418

Results given as pg/ml interferon gamma in culture supernants. Medium acts as a negative control, Ova- ovalubumin, irrelevant control, CEA – test antigen, ConA- nonspecific positive control.

Transgenic CEA animals: CEA transgenic animals are required for the completion of our studies. These animals have been obtained from the National Cancer Institute and are being bred in UAB's Transgenic Breeeding Core Facility. We developed an assay for identification of transgenic progeny, since, for technical reasons, experimental animals are bred from heterozygous males. This assay uses stool samples from the pups in a commercially based immunodetection assay. Samples are homogenized in phosphate-buffered saline, pelleted and the supernatant is used in a Microwell CEA EIA strip (Syntron Bioresearch). The assay is done in an ELISA format with purified CEA protein standard curve. Results are obtained using by reading absorbance at 450 nm on a plate reader. We have found this to be a reliable and rapid assay for identifying transgenic animals.
In the table below, Tg- transgenic animals; Nl-normal animals; high control and low control are provided by Syntron as positive controls, and spiked serum and media also served as positive controls.

Corrected mean OD	
diluent	-0.025
High control	0.843
Low control	0.044
Tg-1	2.848
Tg-2	2.901
Tg-3	2.493
NI-1	-0.027
NI-2	-0.007
NI-3	0.034
Tissue culture med plus 21 ng/ml CEA	0.364
Normal mouse serum plus 50 ng/ml CEA	0.598

KEY RESEARCH ACCOMPLISHMENTS

1. Developed a mouse DC preparation protocol for reliable production of mouse DCs.
2. Produced a truncated CEA antigen, evaluated in a nontransgenic mouse model of adenocarcinoma, demonstrating equivalency with full length CEA.
3. Obtained and began breeding of CEA transgenic mice, developed assay for detection of transgenics

REPORTABLE OUTCOMES

None

CONCLUSIONS

This first year of research has focused primarily on the development of protocols, reagents and assays for completion of the proposed studies. Difficulties were encountered in devising a mouse DC preparation protocol, but we believe these have been overcome. Assays for evaluation of the immune response have also been optimized, and thus we expect immune response analysis to be efficiently evaluated in the coming year. We do not anticipate that our plans for the coming year will differ significantly from those outlined in the grant application.

REFERENCE

1. Tillman BW. Hayes TL. DeGruijl TD. Douglas JT. Curiel DT. Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based vaccination against human papillomavirus 16-induced tumor cells in a murine model. Cancer Research. 60:5456-63, 2000.